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Characterization of interactions between organotin compounds and human serum albumin by capillary electrophoresis coupled with inductively coupled plasma mass spectrometry

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ABSTRACT

Thermodynamic data such as the binding constants are vital parameters describing interactions between exotic trace compounds and biomolecules in biochemical property modeling. In this study, the stability constants of organometallic compound and protein complexes were studied by using capillary electrophoresis coupled with inductively coupled plasma mass spectrometry (CE-ICP-MS), considering its low detection limits and low sample demand. Four organotin compounds (trimethyltin (TMT), tripropyltin (TPrT), tributyltin (TBT), triphenyltin (TPhT)) and human serum albumin (HSA) were used as model organometallic compounds and protein, respectively. Affinity capillary electrophoresis (ACE) and nonequilibrium capillary electrophoresis assays of equilibrium mixtures (NECEEM) were performed and compared by using ICP-MS as the detector to determine the binding constants of organotin compounds and HSA in 1:1 molar ratio assumption. Constant measurements of the two methods were both simple, however, ACE assays were more accurate and more appropriate for the constant determination of the organotin–HSA complexes, considering the errors of the NECEEM method. A good precision of the binding constants ($\log K_b$) using the ACE method was proved by different mathematical calculations, and the values were 6.13 ± 0.51 (TMT), 5.72 ± 0.38 (TPrT), 5.68 ± 0.34 (TBT), 6.05 ± 0.38 (TPhT) respectively for each of the organotin–HSA complexes, showing non-covalent interaction between organotin compounds and HSA. Meanwhile, this study also confirms the suitability of CE-ICP-MS method for further studies on organometallic complexation.

1. Introduction

Non-covalent molecular complex is an important part in regulatory biological processes, such as signal transduction, gene expression and the immune response [1–4]. Non-covalent interactions between biological molecular and exotic compounds, such as trace metals and drugs, have been aroused great interests in biology and medicine studies. Most metallic compounds could form non-covalent complexes with macromolecules in the biological fluid and organs. As one kind of biological molecules, proteins are important parts of organisms and participate in virtually every process within cells. The interaction between proteins and metallic compounds has been considered as an important aspect in the biological study, which does not only regulate the uptake and accumulation of the metallic compounds in the human body but also determine the overall distribution, excretion, differences in efficacy, and toxicity. Metallothionein (MT) is one kind of metal binding protein synthesized primarily in the liver and kidneys, which provide protection against metal toxicity and oxidative stress. Besides, metal ions and compounds could be used as the active sites in the metalloproteins, working as biocatalysts for the reactions during metabolism and so forth. The binding parameters such as dissociation constants, binding constants and stoichiometry are important to describe the non-covalent interactions. Hence, the characterization of the binding phenomenon and the determination of binding parameters are essential for the evaluation of bioaffinity of the metallic chemicals and the interactions between proteins and these compounds.

As a group of the organometallic compounds, organotin compounds were widely applied as PVC stabilizers, pesticides and additives in many industrial and chemical areas [5]. The extensive applications of organotin compounds have resulted in extensive distribution of these compounds in environmental matrixes and various biological samples such as human blood, urine, liver and hair. It is well known that the organotin compounds could cause damage to the neurological system and sexual system. In addition, tributyltin (TBT) and triphenyltin (TPhT) could interfere with

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the cytotoxic function, which have association with the cancer incidences. Although the toxicity of organotin compounds has been well established, there was still a lack of data concerning on the kinetics and the toxicological mechanism of the interaction between organotin compounds and the biomolecules. HSA is a large globular protein with a good essential amino acid profile and it is one of the most abundant proteins in human plasma. In its three-dimensional structure, it bears some drug binding sites which show high selectivity to exotic chemicals and also contributes significantly to the transport of many endogenous and exogenous ligands [6]. Thus, HSA is an important plasma protein in blood and may determine fate, disposition, metabolism, and the toxicity of small chemical compounds in human bodies [7,8]. The investigation of interactions between organotin compounds and HSA were important to illuminate the mechanism of the transportation and biological function of organotin compounds in human bodies. However, no comprehensive studies on the interactions and the kinetics of organotin compounds with HSA have been reported elsewhere.

Nowadays, several techniques have been developed to qualitatively and quantitatively study the binding of organometallic compounds to proteins such as HSA and transferrin protein [9–11]. Among these different methods available for the biomolecular interaction analysis, capillary electrophoresis (CE) has emerged as a powerful technique for quantification of binding interactions. Compared with traditional biological binding assays and NMR techniques, CE requires little sample volume and the operation and separation procedures could be achieved under physiological conditions to avoid the impact on weak interaction of biomolecules in denature conditions. Besides, CE could be used not only for the determination of the binding constants or relative affinity, but also the determination of interaction kinetics is possible. Among the various biomolecular non-covalent interaction investigation methods, ACE and NECEEM methods are the most commonly used methods. ACE is fast and low-consuming for studying molecular interactions. It is performed by dissolving one of the interactants in varying concentrations in the running buffer to observe the changes of the other substrate's migration mobility. ACE method can be performed in physiological solutions to preserve biological interactions between chemical compounds and biomolecules, and is thus more promising than the techniques operated in denaturing mode. In addition, ACE does not suffer from the complication resulting from two-phase chromatographic systems and is therefore particularly suited to study complex formation reactions [12]. NECEEM is another analytical approach, which could also be used to determine the binding constants of the non-covalent interaction between biomolecules and compounds. The method was first introduced to study of protein–probe and DNA–protein interaction in 2002 [4,13,14]. In NECEEM method, a short plug of the equilibrium mixture was introduced into the capillary and subjected to electrophoresis under non-equilibrium conditions. Kinetic parameters such as binding constant (K_b) and monomolecular rate constant (k^{-1}) could be determined in a single experiment which requires only a little amount of the protein in a few minutes [15–17].

Due to the much lower detection limits, CE-ICP-MS system is more attractive for the study of covalent and non-covalent organometallic compounds–protein complexes at trace level compared with CE-UV technique. CE-ICP-MS has already been used in various studies of the interaction of metal drugs and other metal compounds, such as mercury species, with protein or other biomolecules [10,18–20]. However, despite the extensive use, CE-ICP-MS has never been explored to investigate the non-covalent interactions of xenobiotic heavy metals with HSA by using ACE and NECEEM methods. In this study, the applications of ACE and NECEEM were tested and compared to investigate the binding constants of the interaction between the four organotin compounds (TMT, TPrT, TBT, and TPhT) and HSA, while using ICP-MS as an on-line detector.

2. Materials and methods

2.1. Chemicals and reagents

HSA (96–99%, fraction V) was purchased from Sigma–Aldrich (St. Louis, MO). A protein stock solution of 5 mg mL^{-1} was prepared by dissolving 20.0 mg HSA into 4 mL distilled deionized water (DDW) (18 MΩ cm), which was obtained from Milli-Q Advantage A10 system (Millipore, Bedford, MA). Working solutions of HSA were prepared by serial dilution of the stock solution with the phosphate buffer. The phosphate buffer (10×, pH = 7.4) used for incubation was purchased from Sigma–Aldrich (St. Louis, MO).

Trimethyltin chloride (TMT, 99% purity), tributyltin chloride (TBT, 97% purity), tripropyltin chloride (TPrT, 98% purity) and triphenyltin chloride (TPhT, 96% purity) were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Sodium dihydrogen phosphate dihydrate ($NaH_2PO_4{\cdot}2H_2O$) was from Beijing Chemical Co. (Beijing, China); ammonium acetate (NH_4Ac) and disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4{\cdot}12H_2O$) was from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). All the reagents were at least analytical grade. The electrolyte buffer of phosphate was filtered through a 0.22 μm membrane and degassed in ultrasonic bath before use.

2.2. Instrumentation

The schematic diagram for the instrument of CE-ICP-MS system has been published elsewhere [10,19,21]. In brief, the separations of the ACE and NECEEM methods were operated on HP^{3D} CE system (Agilent, Germany). Bare fused-silica capillaries (50-cm long × 75-μm i.d.) used in the experiment were obtained from Yongnian Optical Fiber Company (Hebei, China). The sample solution was introduced into the capillaries by using a hydrodynamic method under a gas presser of 20 mbar for 5 s. Separations were performed under a positive voltage of 20 kV. Every new capillary was conditioned by flushing with 1 mol L^{-1} NaOH for 60 min, 0.1 mol L^{-1} NaOH for 60 min, DDW for 10 min, and then, electrolyte running buffer for 60 min. Prior to each separation, the capillaries were flushed with 0.1 mol L^{-1} NaOH and electrophoresis buffer for 2 min, separately.

An Agilent 7500ce ICP-MS (Agilent, USA) was coupled with the HP^{3D} CE system for the analysis. A MicroMist nebulizer (GE, Australia) was used with a nominal flow rate of 0.1 mL min^{-1}. The plasma gas flow rate was 15 L min^{-1}; carrier gas flow rate was 0.7 L min^{-1} and the makeup gas flow rate was 0.4 L min^{-1}. The plasma RF power was 1500 W. Signals from the four isotopes (^{117}Sn, ^{118}Sn, ^{119}Sn, ^{120}Sn) of tin were monitored in Full-Quant mode (Table 1).

Interfaces of CE coupled with ICP-MS were considered as a key part in the hyphenation technique. Detailed diagram of interface used in this study could be found elsewhere [21]. Briefly, a Pt electrode, a sheath flow and the CE capillary were introduced into the interface based on a cross design. The Pt electrode was grounded. The sheath flow was used to wet the outlet of capillaries and satisfy the demands of closing the electrical circuit from CE. In addition, the sheath flow was also used to transport the outlet of CE capillaries to the MicroMist nebulizer by self-aspiration. To minimize the diffusion of sample zone, the CE capillary was inserted to the end of the nebulizer through a 1/16-in. PEEK tube. 10 mM NH_4Ac solution was used as the sheath flow. The experimental conditions of the CE-ICP-MS hyphenated technique have been listed in Table 1.

Table 1
Summary of experimental parameters.

	Settings
Capillary electrophoresis	
CE voltage	20 kV
CE capillary	Uncoated-fused silica, 75 μm i.d., 50 cm length
Sample injection	Hydrodynamic method under a gas presser of 20 mbar for 5 s
Background electrolyte	10 mmol L^{-1} phosphate buffer (pH = 7.4) (NECEEM); 10 mmol L^{-1} phosphate buffer (pH = 7.4) containing various concentrations of HSA (ACE)
ICP-MS	
Nebulizer	MicroMist nebulizer
Plasma gas flow rate	15 mL min^{-1}
Carrier gas flow rate	0.7 mL min^{-1}
Make up gas flow rate	0.4 mL min^{-1}
RF power	1500 W
Make-up liquid	10 mmol L^{-1} NH_4Ac
Make-up flow rate	100 μmL min^{-1}
Isotope monitored	^{117}Sn, ^{118}Sn, ^{119}Sn, ^{120}Sn
Peak pattern	Full-Quant

2.3. ACE experiments

The experiments were consisted in measuring the variation of organotin compounds in electrophoretic mobility with the increasing concentrations of HSA proteins in the separation electrolyte. In the ACE separation study, the capillary was filled with 10 mM phosphate buffer (pH = 7.4) containing various concentrations of HSA (0–6 μmol) as additive. The separations were performed at a voltage of 20 kV at 20 °C after optimization. Organotin compound solution (200 ppb, as Sn) was injected into the capillaries. The binding of the four organotin compounds with HSA was investigated by monitoring mobility shifts of organotin compounds in CE assays using ICP-MS as the detector. The migration time of neutral marker (DMSO, dimethyl sulfoxide) was calculated as the electroosmotic mobility by UV detector (214 nm).

2.4. NECEEM assays

NECEEM method was also performed on the HP^{3D} CE with ICP-MS as the detector. Unmodified fused-silica capillaries (50 cm long × 75 μm i.d.) were used as the separation capillaries. The voltage of NECEEM method was 20 kV. The separation cartridge temperature was 20 °C. The electrophoresis buffer was 10 mM phosphate buffer (pH = 7.4). After incubation, samples were injected into the capillaries at 20 mbar for 5 s.

For NECEEM measurements, samples containing 1 μmol L^{-1} HSA and 0.1 mg L^{-1} (as Sn) organotin compounds were first incubated at 37 °C for 12 h before injected into the separating capillaries. To simulate physiological conditions, the 10 mmol L^{-1} phosphate buffer containing almost 100 mmol L^{-1} NaCl at pH = 7.4 was used as the incubation solution to conduct the binding experiments. After incubation, the mixture of organotin compounds and HSA was obtained and used for determine the binding constants.

3. Results and discussion

HSA (pI = 4.9) was negative charged in the phosphate buffer (pH = 7.4) and the four organotin compounds are +1 charged. Same to the other metal ions or metal compounds, organotin compounds could achieve equilibrium within the ACE separation time [22,23]. Usually, the interaction between drugs and biomolecules could be considered as 1:1 molar ratio in CE analysis [24–26]. Comparing to the organotin compound concentration, the maximum protein concentration is not high. During each separation, it could be assumed that only tin compound and the complex exist in the running buffer. Additionally, in the previous published papers, CE-ICP-MS has been applied successfully to investigate the stability constant determination of lanthanum-oxalate and uranyl-oxalate complexes in the assumption of 1:1 metal-to-ligand stoichiometry [10,27]. Thus, for simple calculation, the interaction of protein–organotin compound was assumed to be 1:1 complex molar ratio in described study firstly. In this case, the binding constant could be described as follows:

$$K_b = \frac{[C]}{[P][L]} \quad (1)$$

where K_b, $[C]$, $[P]$ and $[L]$ represent the binding constant, the concentration of the complex, free protein and free ligand, respectively in the reaction equilibrium.

3.1. Experimental optimization

The diagram of the interface of CE-ICP-MS used in the experiment has been published elsewhere [21]. As previously illuminated, analytical conditions have to be fit for metal complex stability constant determination. Thus, phosphate buffer was used as the separation electrolyte, which also could offer good resolution. Under physiological condition (pH = 7.4), HSA bears a negative net charge (pI = 4.9). Though positive charges are still present on the free amine moieties of the protein, adsorption of HSA on the negatively charged inner surface of the bare silica capillary could be minimized. Therefore, pH was chosen to be 7.4 to simulate the physiological conditions in this experiment.

The ionic strength could affect the stability constant calculation. And the current intensity of CE-MS should be no more than 50 mA. So, in order to simplify the ionic strength correction in the stability constant calculation, the ionic strength of the back ground electrolyte should be in a low value, which also should provide efficient separation. However, the separation efficiency is poor while the concentration of electrolyte is 5 mM. Thus, a 10 mM of phosphate buffer was chosen for the background separation electrolyte and the current intensity is 30 mA under the optimized condition. The effect of separation voltage (5, 10 and 20 kV) for the analyte separation has also been investigated. The lower voltages (5 and 10 kV) resulted in co-elution of each organotin compound and the corresponding complex. So we finally chose the separation voltage of 20 kV, which could provide a good separation of the analytes. The analytical conditions have also been summarized in Table 1.

3.2. ACE assays

In ACE method, migration parameters such as time and area of interacting molecules are used to quantify and identify the specific binding and estimate binding constants. In capillary electrophoresis, the mobility of ionic species (μ) is directly related to velocity (ν), or time of migration (t) according to the following equation:

$$\mu = \frac{\nu}{E} = \frac{L_{det}L}{tU} \quad (2)$$

where E is the electric field intensity (V cm^{-1}); L_{det} is the capillary length from the capillary inlet to the detector (cm); L is the length of the capillary (cm); U is the voltage applied (V). μ_{app} (the apparent mobility) is the sum of μ_{ep} (the electrophoretic mobility) and μ_{eo} (the electroosmotic mobility) as follows:

$$\mu_{app} = \mu_{ep} + \mu_{eo} \quad (3)$$

μ_{eo} could be evaluated by using dimethylsulfoxide (DMSO) as a neutral marker determined in UV detector, at a wavelength of 214 nm.

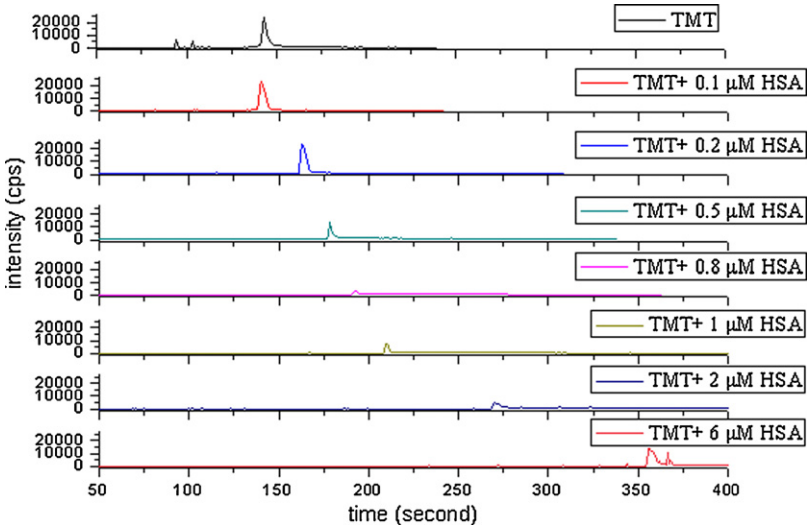

Fig. 1. ACE electrophoregram about the mobility shift of the organotin compound (TMT) obtained with background buffers containing various concentrations of HSA. (Separation condition: uncoated-fused silica (75 μm i.d. × 50 cm); applied voltage: 20 kV; hydrodynamic injection (20 mbar, 5 s); background electrolyte: 10 mmol L^{-1} phosphate buffer (pH = 7.4) containing various concentrations of HSA (0–6 μmol L^{-1})).

While the measurements were carried out by ICP-MS in the CE-ICP-MS online system, the apparent mobility of a metal ion or organometallic compound (M) could be determined according to the following equation:

$$\mu_{app} = \frac{L^2}{t_{MS}(M)U} \quad (4)$$

where $t_{MS}(M)$ is the migration time of a metal ion or organometallic compound (M).

In the ACE mode, the effective mobility (μ_i) of the organotin compounds could be obtained by the weighted average of all the individual species electrophoretic mobilities, including the electrophoretic mobility of free organometallic ion ($\mu(M_{free})$) and the electrophoretic mobility of organometallic complex ($\mu(ML)$). Compared with the complex formation constant, the expression of binding isotherm could be expressed as:

$$\frac{\mu_i - \mu(M_{free})}{\mu(ML) - \mu_i} = K_b[P] \quad (5)$$

The binding constant and actual mobility of the complex could be obtained by measuring the shift in migration time of the organotin compounds according to the different known protein concentration contained in the electrolyte. In the above equation, because of the low concentration of organotin injected or the low stability constant, the concentration of free protein could be replaced by the concentration of total protein. There are also three other equations which could be used to calculate the binding parameters (K_b and $\mu(ML)$) as follows:

$$\frac{1}{\mu_i - \mu(M_{free})} = \frac{1}{K_b(\mu(ML) - \mu(M_{free}))} \times \frac{1}{[P]} + \frac{1}{\mu(ML) - \mu(M_{free})} \quad (6)$$

$$\frac{[P]}{\mu_i - \mu(M_{free})} = \frac{1}{\mu(ML) - \mu(M_{free})} \times [P] + \frac{1}{K(\mu(ML) - \mu(M_{free}))} \quad (7)$$

$$\frac{\mu_i - \mu(M_{free})}{[P]} = -K(\mu_i - \mu(M_{free})) + K(\mu(ML) - \mu(M_{free})) \quad (8)$$

Table 2 provided the associations between the weighted linear regressions and the binding constants or the effective mobility of the organotin compounds mentioned in above four equations.

In order to examine the interaction between the organotin compounds and HSA, the shifts of CE mobility have been monitored. The concentration of HSA was varied from 0 to 6 μmol L^{-1} in the experiments. The electrophoretic mobilities of the organotin compounds or the organotin-complexes were deduced from the μ_{app} by subtracting the electroosmotic mobility determined by UV detection using DMSO as neutral marker, which was also named as "double detection". Shown in Fig. 1, the binding profile clearly indicated that the migration times of the organotin compounds were changed with the increasing concentrations of HSA. For instance, the migration time of TMT increased from 138 s (0 μmol L^{-1}) to 355 s (6 μmol L^{-1}). The increasing mobility shift of the organotin compounds indicated that dynamic, reversible and non-covalent interaction existed between the four organotin compounds and HSA.

The experimental data were obtained in triplicated for each point in Fig. 2, which showed the curves obtained by ICP-MS detector. The diagram showed the measured electrophoretic mobilities of TMT vs HSA concentration in the range of 0–6 μmol L^{-1}. It was observed that the electrophoretic mobilities of TMT decreased from 6×10^{-4} ($cm^2\ V\ s^{-1}$) to 3×10^{-6} ($cm^2\ V\ s^{-1}$). The theoretical curves of the electrophoretic mobilities of TMT (Fig. 2) also indicated the presence of interaction between organotin compounds with HSA. As the assumption of the molar ratio of organotin compounds and HSA was 1:1, the equilibrium binding constant (K_b) of the organotin compound–HSA complex calculated by the four linear regressions (Table 3) achieved a good agreement between the different four linear regressions. The values of $\log(K_b)$ obtained by the four linear regressions were almost the same for the interactions of each organotin compound and HSA, considering the related uncertainties. The average values of the $\log(K_b)$ were 6.13 ± 0.51 (TMT), 5.72 ± 0.38 (TPrT), 5.68 ± 0.34 (TBT), 6.05 ± 0.38 (TPhT). These average values were less than 7, also less than the values of mercury

Table 2
Linear regressions of the binding isotherm in the case of 1:1 complexation for the determination of binding constants.

Method name	Plotting method	K_b
Isotherm	$(\mu(ML) - \mu_i)/(\mu_i - \mu(M_{free}))$ vs $[P]$	Slope
x reciprocal	$(\mu_i - \mu(M_{free}))/[P]$ vs $\mu_i - \mu(M_{free})$	−Slope
y reciprocal	$[P]/(\mu_i - \mu(M_{free}))$ vs $[P]$	Slope/intercept
Double reciprocal	$1/(\mu_i - \mu(M_{free}))$ vs $1/[P]$	Intercept/slope

Table 3
The equilibrium binding constant K_b of the four organotin compounds–HSA complexes obtained with the different linear regressions, and associated uncertainties (ACE).

	Mobility ratio difference method ($\log(K_b)$)	*x*-Reciprocal ($\log(K_b)$)	*y*-Reciprocal ($\log(K_b)$)	Double-reciprocal ($\log(K_b)$)
TMT–HSA	6.30 ± 0.20	5.53 ± 0.23	6.70 ± 0.40	6.00 ± 0.22
TPrT–HSA	6.00 ± 0.14	5.60 ± 0.17	5.65 ± 0.29	5.61 ± 0.57
TBT–HSA	6.00 ± 0.14	5.47 ± 0.45	5.49 ± 0.15	5.75 ± 0.15
TPhT–HSA	6.30 ± 0.22	5.75 ± 0.14	6.50 ± 0.06	5.65 ± 0.05

Table 4
The equilibrium binding constants K_b of the four organotin compounds–HSA complexes obtained with the different linear regressions (NECEEM).

Complexes	The equilibrium binding constants $\log(K_b)$
TMT–HSA	7.02 ± 0.12
TPrT–HSA	7.25 ± 0.08
TBT–HSA	7.29 ± 0.06
TPhT–HSA	7.37 ± 0.16

species–HSA complexes [10], which means the binding is not as much stable than the mercury species with HSA.

3.3. NECEEM assays

NECEEM assay was another analytical approach of quantitative analysis of peak profiles to determine the equilibrium binding constants by using CE-ICP-MS. In the NECEEM method, the ligand dissociated from the complex during the separation could be detected as a bridge between the complex peak and free ligand peak. As described previously, the binding isotherm could be determined using following equation (Table 4):

$$K_b = \frac{1 + ((A_C + A_{AC})/A_A)}{[P]_0(1 + (A_A/(A_C + A_{AC}))) - [A]_0} \quad (9)$$

where A_C, A_{AC}, and A_A are the peak areas of the complex, dissociating part of complex, and organotin compounds, while $[P]_0$ and $[A]_0$ are the known initial protein and organotin compound concentrations [28].

There are non-linear and linear regression methods for the data treatment. In this study, linearization methods were preferred for the mathematical treatment of experimental data to avoid the high error levels made by the non-weighted linearization. Fig. 3 showed the electrophoregram of the TMT–HSA complex in the separation condition introduced previously. In the method of NECEEM, the organotin compounds dissociated from the complex during the separation of CE and the concentrations decreased exponentially when out of equilibrium, which was considered as a bridge between the organotin–HSA complex and the free organotin compounds peak. The areas A_A, A_C, and A_{AC} were calculated as demonstrated from the figures. The equilibrium binding constants ($\log K_b$) for each organotin compound interacting with HSA were calculated using Eq. (9) and equaled to 7.02 ± 0.12 (TMT), 7.25 ± 0.08 (TPrT), 7.29 ± 0.06 (TBT), 7.37 ± 0.16 (TPhT). The reproducibility of NECEEM-ICP-MS was also acceptable. The relative standard deviation (RSD) values ranged from 0.8% to 2.2% (n = 3), which indicated the precision of NECEEM was also good for studying the interaction of organotin compounds with HSA by using ICP-MS as the detector.

3.4. Comparison of ACE assays and NECEEM assays

In this study both ACE and NECEEM methods were applied to determine the thermodynamic data of the binding of the four organotin compounds and HSA. ACE is a widely used analytical technique while using macromolecules such as protein or other biological molecules, as buffer additives. Coupling with ICP-MS, the method examined the interaction between the metallic compounds and the biomolecules in trace concentrations of the metal species. Herein, the ACE method has provided a direct evidence of non-covalent interaction of the four organotin compounds (TMT, TPrT, TBT, TPhT) with HSA. The binding constants of organotin compounds and HSA also have been figured out by the

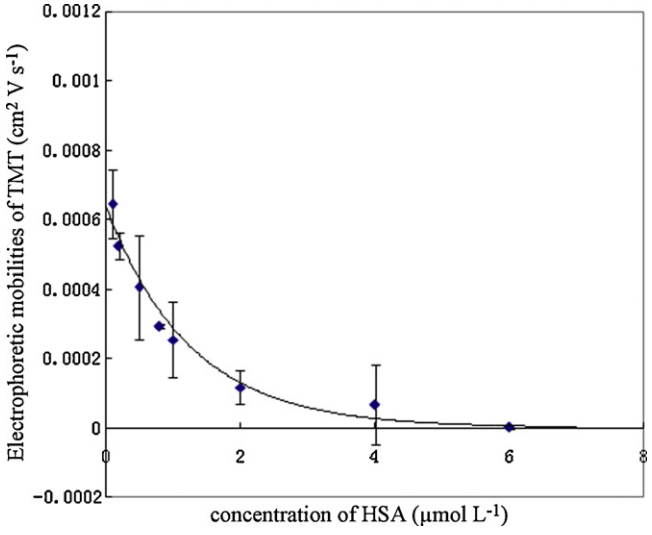

Fig. 2. The electrophoretic mobilities of organotin compound (TMT) measured vs HSA concentration. (Separation condition: uncoated-fused silica (75 μm i.d. × 50 cm); applied voltage: 20 kV; hydrodynamic injection (20 mbar, 5 s); background electrolyte: 10 mmol L^{-1} phosphate buffer (pH = 7.4) containing various concentrations of HSA (0–6 μmol L^{-1})).

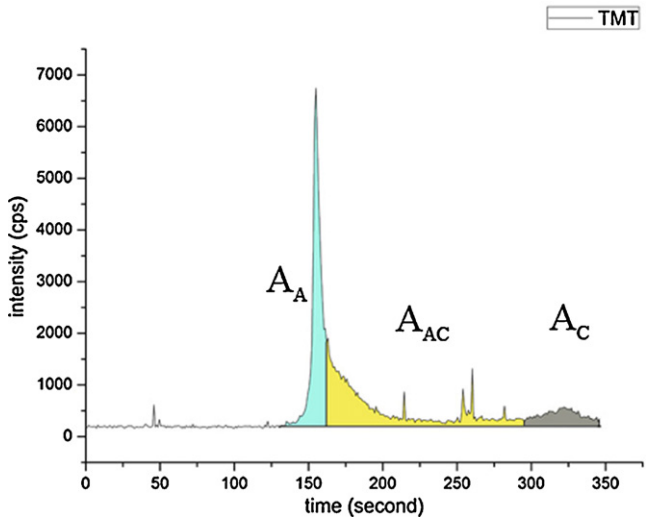

Fig. 3. Electrophoregram of the organotin compound (TMT)–HSA complex by the method of NECEEM. (Separation condition: uncoated-fused silica (75 μm i.d. × 50 cm); applied voltage: 20 kV; hydrodynamic injection (20 mbar, 5 s); background electrolyte: 10 mmol L^{-1} phosphate buffer (pH = 7.4)).

mathematical equations. Besides, four linear regressions were managed to figure out the binding constants for accuracy. The RSD values (6.0–8.3%) of the binding constants for the interaction between organotin compounds and HSA indicated that precision of the method was accurate in investigating the interaction between organotin compounds and HSA. The obtained binding constants ($\log K_b$) of organotin compounds and HSA using ACE is in the range of 5.68–6.17, which is less than the binding constants of the mercury species with HSA (7.00–7.11) [10]. That means the binding stability between organotin compounds with HSA is not as stable as that of the Hg–HSA binding. These values also indicated the bindings between organotin compounds and HSA are reversible and non-covalent. It also elucidated that adducts of organotin compounds with HSA is more likely to dissociate during the electric field in the separation. However, the interactions of organotin compounds with HSA are relatively stronger than the platinum metallodrug (KP1019), whose binding constant ($\log K_b$) was 4.02 and less than the values of organotin compounds [29]. Results showed that the binding of organotin–HSA complexes were weaker than that of mercury–HSA complexes. So, the tin compounds in the complexes could be replaced by other metal compounds, such as mercury compounds. Organotin compounds would be likely to bind to HSA to be transported after imported in blood stream and translocated into the organs in human body. Organotin compounds could accumulate in livers and tissues, resulting in sex differentiation or other illnesses. Organotin compounds have already been considered as endocrine disruptors [30,31]. However, the mechanism for the interaction between organotin species with proteins and other biomolecules is not clear and still needs to be elucidated.

NECEEM assay is simpler to allow equilibrium and kinetic constants determination in a single experiment. In addition, considering the RSD values of the binding constants were in the range of 0.8–2.2%, the reproducibility of NECEEM-ICP-MS was also acceptable. However, the values of the binding constants of the four organotin compounds with HSA are bigger than the values of mercury–HSA complexes, which is incompatible with the phenomenon in the experiments. Organotin–HSA complexes were more likely to break up during the separation than mercury–HSA complexes according to the bridge area in the electropherotogram, which also means the interactions were reversible. So, the binding of organotin–HSA complexes were more fragile than mercury–HSA complexes. Normally, K_b of reversible reactions is in the range of 10^{-7}–10^{7} [32–35]. Thus, the binding constant values of organotin–HSA complexes could be less than 7 ($\log K_b$). Other important impacts might also affect the measured $\log K_b$ values. For example, HSA is likely to be adsorbed onto the inner wall of the capillaries, which could affect the accuracy of the NECEEM method. In addition, the peak tailing could also affect the mathematical analysis of the areas of the free organotin compounds and the bridge. Hence, NECEEM method might not be suitable for the determination the equilibrium binding constants of organotin compounds and HSA by using ICP-MS as the detector.

4. Conclusions

In this work, we demonstrated the online CE-ICP-MS system to be a useful method for investigating the interaction between organotin compounds (TMT, TPrT, TBT, and TPhT) and HSA. Comparisons of ACE and NECEEM assays coupled with ICP-MS were processed to determine the binding constants of the organotin–HSA complexes. In the ACE assays, mathematical calculations of the binding constants were compared to obtain a good accuracy. The RSD values of ACE assays were in the range of 6.0–8.3%, which indicated the precision was good. Although the NECEEM method provided a more simple way to figure out the stability constants, however, ACE assays were more suitable for the determination of the stability constants of interactions between the organometallic compounds and biomolecules. Furthermore, the binding constants ($\log K_b$) of the four organotin compounds–HSA complexes determined by ACE assays were 6.13 ± 0.51 (TMT), 5.72 ± 0.38 (TPrT), 5.68 ± 0.34 (TBT), 6.05 ± 0.38 (TPhT). The developed method could also be applied for the investigation of the interactions between other metal ions or metal species and biomolecules.

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